**A DISSERTATION ON**

# CORRELATION OF CT LIVER DENSITY WITH SERUM FERRITIN IN TRANSFUSED THALASSEMIA MAJOR CHILDREN

**M.D (BRANCH VII)**

**PAEDIATRIC MEDICINE**

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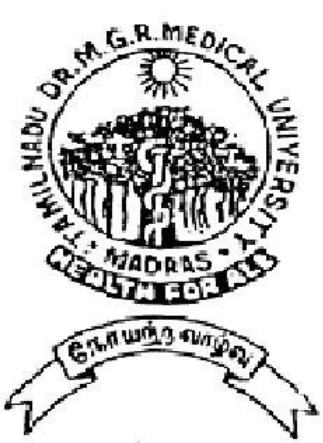


## THE TAMILNADU DR.MGR.MEDICAL UNIVERSITY CHENNAI, TAMILNADU

# CERTIFICATE

This is to certify that the dissertation titled **"Correlation of CT liver density with serum ferritin in transfused thalassemia major children"** submitted by **Dr.M.Ramya** to the Faculty of pediatrics,The Tamilnadu M.G.R.Medical University,Chennai in partial fulfillment of the requirement for the award of M.D.Degree (Pediatrics) is a bonafide research work carried out by her under our direct supervision and guidance.

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# DECLARATION

I **Dr.M.Ramya**, solemnly declare that the dissertation titled "**Correlation of CT liver density with serum ferritin in transfused thalassemia major children"** has been prepared by me.

This is submitted to the **Tamilnadu Dr.M.G.R.Medical University**, Chennai in partial fulfillment of the rules and regulations for the M.D.Degree Examination in Paediatrics.

Place: Madurai

Date: **Dr.M. RAMYA**

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# CONTENTS

# INTRODUCTION

Thalassemias represent the most common single gene disorder known. In many parts of the world, they constitute major public health problems. It occurs most frequently in people of Italian, Greek, Middle eastern, Asian and African ancestry.

Beta Thalassemia is one of the commonest disorder affecting Indians.The incidence of Thalassemia is very high,over 250 million people carrying the defective gene and carrier frequency varies from 3-17% in various populations.Ten thousand Thalassemic children are born every year in India.A higher frequency is noted in certain communities like Sindhis,Panjabis,Baniyas from Gujarat and Gouda from Karnataka.

It was first described by Thomas cooley and Pearl Lee in children of Italian origin[1].Because all early cases were reported in children of Mediterranean origin,the disease was later termed Thalassemia from the greek word for sea,"thalassa"[2].

The thalassemia syndromes are a heterogeneous group of inherited anemias characterized by defects in the synthesis of one or more of the globin chain subunits of the hemoglobin tetramer.

The most common forms of thalassemia arise from total absence of structurally normal globin chains or a partial reduction in their synthesis. In contrast to the structural hemoglobinopathies (e.g., sickle cell anemia), which are characterized by the production of normal amounts of mutant globin chains having deranged physical or chemical properties, thalassemias are quantitative disorders: the primary lesion lies in

the amount of globin produced. However, some rare forms of thalassemia are characterized by the production of structurally abnormal globin chains in reduced amounts. These thalassemic hemoglobinopathies share features of both thalassemia and structural hemoglobinopathies.

If the synthesis of alpha chains is suppressed, level of all three normal hemoglobins-A1,A2 and F are reduced (alpha Thalassemia). If beta chains are suppressed because of mutation in beta gene then production of adult hemoglobin is suppressed (beta thalassemia) .

Two genes control the production of beta globin.Mutations of one or both of them can cause the disorder.There are three main forms of beta thalassemia.The severity of the condition is determined by whether one or both globin genes are mutated and by the severity of mutation.

By the age of 6 to 12 months, most affected infants show pallor, irritability, growth retardation, jaundice, and hepatosplenomegaly as a result of extramedullary hematopoiesis. By 2 years of age, 90% of infants are symptomatic, and progressive changes in the facial and cranial bones develop.

More than 80% of children with homozygous thalassemia require regular RBC transfusions by 1 to 2 years of age because of severe anemia. Life expectancy with untreated thalassemia major is less than 5 years. Outcomes of the patients with poorly transfused thalassemia major reflected severe anemia and compensatory hypertrophy of medullary and extramedullary erythroid tissue in attempt to compensate for the anemia.

Many of the features of Thalassemia become less severe with transfusion therapy. But creation of excessive iron stores associated with hemosiderosis is a major concern in individuals with beta Thalassemia and many of the complications of Thalassemia are the result of increased iron deposition from repeated blood transfusions.Iron released from the breakdown of endogenous or transfused RBCs cannot be utilized for hemoglobin synthesis. This iron is eventually deposited (hemosiderosis) in various organs e.g-liver, spleen, heart, endocrine glands leading to their malfunction.. At a total body iron burden of 40 g, organ function begins to fail, and at 60 g or more, intractable cardiac failure has its onset.

The liver is a major repository of transfused iron.Hepatic parenchymal iron accumulation, demonstrated after only 2 years of transfusion therapy, may rapidly result in portal fibrosis in a significant percentage of patients. One center has observed portal fibrosis in a high percentage of biopsies in children under the age of 3 years. In young adults with thalassemia major, in whom liver disease remains a common cause of death, viral infection may act synergistically with iron in accelerating the development of liver damage. Managing iron overload in Thalassemia syndromes require a reliable assessment of excessive iron overload and organ iron distribution.

Measurement of hepatic iron concentration is the most quantitative, specific, and sensitive method for determining the body iron burden in patients with thalassemia major. Liver biopsy permits chemical measurement of the nonheme (storage) iron concentration and histochemical examination of the pattern of iron

accumulation in hepatocytes and Kupffer cells as well as evaluation of the extent of inflammation, fibrosis, and cirrhosis. These direct methods for assessing iron status have the disadvantages of being invasive procedures, with their attendant discomfort, lack of acceptability to patients, and, in the case of liver biopsy, risk.

Several noninvasive means of measuring tissue iron stores include determination of hepatic magnetic susceptibility, computed tomography (CT), and magnetic resonance imaging (MRI). As serum ferritin is the most commonly used parameter for judging iron overload the present study was done to determine whether sigificant correlation exists between serum ferritn and CT liver density. CT was preferred to MRI in the present study as it is more widely available and less expensive.

# REVIEW OF LITERATURE

## Thalassemia major

Homozygosity for thalassemia genes is usually associated with severe anemia because of a marked reduction of synthesis of the globin chains of HbA. However, reduction of HbA synthesis does not explain the hemolysis and ineffective erythropoiesis that are consequences of unbalanced globin chain synthesis. In homozygous thalassemia, alpha globin chains are produced in normal amounts and accumulate, denature, and precipitate in the RBC precursors in the bone marrow and circulating RBC. These precipitated alpha globin chains damage the RBC membrane, resulting in destruction within the bone marrow (ineffective erythropoiesis) and in the peripheral blood.

By the age of 6 to 12 months, most affected infants show pallor, irritability, growth retardation, jaundice, and hepatosplenomegaly as a result of extramedullary hematopoiesis. By 2 years of age, 90% of infants are symptomatic, and progressive changes in the facial and cranial bones develop. The hemoglobin level may be as low as 30 to 50 g/L.

The natural course of thalassemia major is one of recurrent infections, progressive cachexia, and death by 5 years of age. Aggressive transfusion therapy permits near-normal growth and development in childhood but itself produces progressive organ damage with death from iron overload in adolescence or early adult

life. With transfusion therapy, approximately 25% of patients survived to their mid twenties. Still, nearly two-thirds die with complications of myocardial hemosiderosis at a mean age of 16 to 17 years. The transfusional iron overload is compounded by increased intestinal absorption of iron is suggested by the observation that the iron present at autopsy exceeds that which can be accounted for by transfusions alone. Enhanced iron absorption has been confirmed by direct measurement. The introduction of iron chelation therapy in the 1970s constituted a further therapeutic advance. In one series, the median age of survival was 31 years[3].The most important factors associated with survival were the age at which chelation therapy was introduced and the success with which serum ferritin was maintained below 2500 ng/ml[4].

The symptomatic thalassemia syndromes are predictably complicated by tissue iron toxicity. Avoidance of unnecessary iron exposure is of paramount importance. As in association with other chronic hemolytic anemias, folate supplementation may be necessary.The hepatitis B vaccine is recommended for all patients before starting transfusion therapy, and a polyvalent pneumococcal vaccine should be given if splenectomy is planned.

**Transfusion therapy**

In the past, transfusions were given only for the temporary relief of symptoms caused by severe anemia. This practice was based on the premise that the restriction of blood administration would delay the onset of transfusional hemosiderosis. Because

erythropoiesis was not suppressed, the numerous complications resulting from exuberant erythroid hyperplasia persisted. Survival into the second decade was achieved at the expense of progressive facial and skeletal deformities, osteoporosis, and splenomegaly.

Later, transfusion programs designed to maintain the minimum hemoglobin concentration above 10 g/dl and the mean hemoglobin concentration at 12 g/dl improved the quality of life without accelerating the lethal complications of iron overload[5]. Such hypertransfusion programs effectively suppress erythroid activity and prevent the unlimited bone marrow expansion that underlies the skeletal pathology of thalassemia major.

When begun early in life, transfusions prevent the facial stigmata of thalassemia and facilitate normal growth during the first decade[6]. Growth usually is permanently delayed if normal levels of hemoglobin are not maintained until after the child is three or 4 years of age[7]. Even in optimally transfused patients, growth begins to deviate from the normal curve at 10 to 13 years and subsequent sexual development is delayed, both consequences of iron overload[8]. Additional advantages of a regular transfusion program include prevention or delay in the development of congestive splenomegaly, fewer severe infectious illnesses, and improved cardiac reserve[9]. Fortunately, the concern that a more aggressive transfusion program would accelerate iron overload and lead to death at an earlier age has not been realized. Failure to observe a deleterious impact of the more liberal use of blood on survival is attributed to suppression of excessive gastrointestinal iron absorption by maintenance of a normal hemoglobin concentration.

Because bone marrow mass and blood volume remain expanded with hypertransfusion, a more aggressive program of transfusion (supertransfusion) has been advocated by some[10]. Maintenance of a mean hemoglobin value of 14 to 15 g/dl almost completely suppresses endogenous erythropoiesis, thereby shrinking the bone marrow mass and reducing the blood volume by approximately 20%[11]. Reduction of the blood volume, in turn, permits the maintenance of a higher hemoglobin concentration without an increase in the transfusion requirement. Within 1 to 4 months of raising the minimum hemoglobin value to 12 g/dl, the transfusion requirement returns to that amount necessary to maintain a minimum level of 9 g/dl[12]. The effect of supertransfusion on growth and development during the second decade remains to be evaluated.

The use of young red cells (neocytes) may reduce the total blood requirement. Collection of neocytes by continuous flow centrifugation or by fractionation of conventionally collected blood with a blood cell processor is feasible. Selective removal of the least dense fraction yields a population of cells that have a mean age of 12 to 21 days and a half-survival time of 40 to 47 days in contrast to 29 days for standard frozen cells. Unfortunately, the reduction in blood requirements during extended trials of neocytes is substantially less than predicted[1].

The complete genotype of patient red cells should be determined before the first blood transfusion is given. This information may prove invaluable for identifying compatible blood should antibodies to minor blood group antigens develop. It is recommended that blood be administered through a leukofilter to minimize the risk of

febrile reactions that result from the development of antibodies to white blood cells.

**Iron Chelation**

Most patients with thalassemia major die from complications of iron overload. At a total body iron burden of 40 g, organ function begins to fail, and at 60 g or more, intractable cardiac failure has its onset. In recent years, concerted and aggressive steps have been taken to reckon with this inevitable complication.

The most effective iron chelating agent widely available is deferoxamine, a siderophore produced by Streptomyces pilosus[14]. Experience with the use of this drug for rectifying transfusional iron overload has been reviewed. Iron excretion after the administration of deferoxamine is proportional to body iron stores. In patients with thalassemia major who are begun on regular transfusions in the first year of life, mobilization of clinically significant amounts of iron cannot be achieved with daily intramuscular injections of deferoxamine until five to ten years of age, and negative iron balance is achieved infrequently at any age[15]. Nevertheless, chronic chelation therapy begun in the first decade of life reduces hepatic iron levels, retards the progression of hepatic fibrosis, and facilitates continued growth. Unfortunately, it appears to have little effect on the endocrine and cardiac complications of hemosiderosis[16].

When given by continuous intravenous or subcutaneous infusion, deferoxamine sustains a greater urinary excretion of iron[17]. The greater efficiency of continuous infusions of the drug is thought to relate to the constant exposure of a labile iron pool to the chelating agent. The relationship between iron excretion and deferoxamine infused is

linear to a dose of 1.5 to 2.0 g/24 hours. In some heavily iron-loaded patients, a linear response continues to 4, 8, or even 16 g/24 hours. The requisite amount of drug is given most conveniently with a small portable infusion pump that permits delivery of microliter quantities of the drug subcutaneously from a standard syringe through a butterfly needle placed by the patient in the anterior abdominal wall. The total daily dose is infused over an 8 to 12-hour period at night, allowing freedom from the device during daylight hours. Limited experience with high-dose deferoxamine (6 to 12 g daily) given intravenously suggests that it may be superior to conventional subcutaneous therapy in heavily iron overloaded patients[18].

Institution of chelation therapy before 10 years of age preserves gonadal function and facilitates normal sexual maturation in most patients[1]. Because the major cause of death in thalassemia patients relates to myocardial hemosiderosis, the value of chelation therapy ultimately is determined by its success in preventing or reversing cardiac disease. Although relevant data are limited, chronic deferoxamine infusions appear to prevent or delay the development of heart disease in heavily transfused patients.

Current efforts are directed at the prevention rather than the treatment of iron overload. It is recommended that deferoxamine be started after the first 10 to 20 transfusions, or when the serum ferritin reaches 1000 ng/mL. In general these criteria are reached at about 3 years of age. Infusions started at this age can maintain iron balance. Needle biopsy of the liver with quantitation of liver iron permits a more accurate measurement of body iron burden. Those who have used this approach recommend that

chelation therapy be started as hepatic iron concentration approaches 9 µm iron per gram liver, wet weight, or 1.5 mg iron per gram liver, dry weight. This can occur as early as 1 year after the beginning of regular transfusions.

When given before 3 years of age, deferoxamine must be given at a reduced dose (20 to 30 mg/kg/day) to prevent the drug's adverse effect on linear growth. After 3 years of age, the dose of deferoxamine may be increased to 40 to 50 mg/kg/day. Doses of deferoxamine in excess of 50 to 60 mg/kg/day are associated with visual and auditory neurotoxicity . Sudden deterioration in pulmonary function, possibly immune-mediated, has been observed shortly after the institution of deferoxamine in some patients.

The efficacy of a properly executed program of iron chelation in preventing iron accumulation is well documented.Failure to maintain the serum ferritin below 2500 ng/ mL or the total body iron burden below 1 g/kg were adverse prognostic factors for survival . Heart failure, diabetes mellitus, and early death are prevented when deferoxamine is begun at an early age and given in amounts proportional to the transfusion-related iron load.

Ascorbic acid appears to render tissue iron more accessible to deferoxamine, thereby enhancing total iron excretion without increasing iron absorption. The net amount of iron excreted by iron-overloaded subjects receiving deferoxamine is increased twofold by the daily oral administration of 200 to 500mg ascorbic acid. Unfortunately, ascorbate also increases the toxicity of tissue iron. Sudden deterioration of heavily iron-loaded patients given both deferoxamine and ascorbic acid is well documented.[20]

Deferoxamine is expensive and its administration is so cumbersome as to invite noncompliance. A safe and effective chelating agent that can be taken by mouth is needed badly. Deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one) is the most promising. In a pilot study of patients unable or unwilling to use deferoxamine, oral deferiprone was reported to induce a sustained decrease in body iron. In randomized trials comparing deferiprone and deferoxamine, however, a 50% increase in hepatic iron was observed after 2 years in patients given the oral chelator, whereas no significant accumulation of iron was detected in those receiving deferoxamine. Long term therapy with deferiprone may not provide the needed control of body iron in a significant proportion of chronically transfused patients. Reversible agranulocytosis and arthralgias are infrequent complications of deferiprone.

ICL 670 is an orally active chelating agent developed for the treatment of iron overload.It represents a new class of tridentate iron chelators with a high specificity for iron. Phase III trials are completed. There are no major safety concerns at doses upto 80 mg/kg/day. Iron excretion is dose dependent and is almost entirely in the feces.The plasma half life (11-19 hours) supports the once daily oral dosing regimen.

**Laboratory Evaluation of Iron Status**

Body iron supply and stores may be evaluated by both direct and indirect means, but no single indicator or combination of indicators is ideal for the evaluation of iron status in all clinical circumstances. As body iron content decreases from the iron-replete

normal to the amounts found in iron deficiency anemia, or increases to the magnitudes found in iron overload, each available measure reflects in a different manner the continuum of changes shown in. In addition, each indicator may be affected by other conditions, such as infection, inflammation, liver disease, malignancy, or malnutrition, and must be interpreted with an awareness of the potential influence of such coexisting disorders.

**Direct Measures**

The direct measures of body iron status yield quantitative, specific, and sensitive determinations of body or tissue iron stores. Quantitative phlebotomy provides a direct measure of total mobilizable storage iron. Repeated venesection to remove about 500 ml of blood weekly is performed until the hemoglobin concentration falls to <10 g/dl for 2 weeks without further phlebotomy. Mobilizable storage iron may then be calculated as the amount of hemoglobin iron removed, with corrections for the hemoglobin deficit and estimated GI iron absorption during the course of phlebotomy. Quantitative phlebotomy is inapplicable to most anemic disorders but is occasionally useful in the diagnostic evaluation of some forms of iron overload, for example, in patients with hereditary hemochromatosis who do not undergo liver biopsy.

Bone marrow aspiration and biopsy can provide information about (1) macrophage storage iron, by semiquantitative grading of marrow hemosiderin stained with Prussian blue, or if needed, by chemical measurement of nonheme iron; (2) the iron

supply to erythroid precursors, by determining the proportion and morphology of marrow sideroblasts (i.e., normoblasts with visible aggregates of iron in the cytoplasm); and (3) the general morphologic features of hematopoiesis. Bone marrow aspiration and biopsy are useful in studies of iron deficiency but of limited applicability in the evaluation of iron overload because no information about the extent of parenchymal iron deposition is provided.

In the evaluation of iron overload, liver biopsy is the best direct test for assessing iron deposition, permitting quantitative measurement of the nonheme iron concentration and histochemical examination of the pattern of iron accumulation in hepatocytes and Kupffer cells as well as evaluation of the extent of inflammation, fibrosis, and cirrhosis.

These direct methods for assessing iron status have the disadvantages of being invasive procedures, with their attendant discomfort, lack of acceptability to patients, and, in the case of liver biopsy, risk. Several noninvasive means of measuring tissue iron stores include determination of hepatic magnetic susceptibility, computed tomography (CT), and magnetic resonance imaging (MRI).

**Indirect Measures**

The indirect measures of body iron status have the advantages of ease and convenience, but all are subject to extraneous influences and lack specificity, sensitivity, or both. The measurement of plasma ferritin provides the most useful indirect estimate of body iron stores.

**Twenty-four-hour deferoxamine-induced urinary iron excretion**

The usefulness of measurement of the amount of chelated iron in the urine induced by a single intramuscular dose or prolonged subcutaneous infusion of deferoxamine has several limitations in the accurate assessment of body iron burden. Most important is the poor correlation between urinary iron excretion and hepatic iron concentration, in part because the relative amounts of iron excreted into stool and urine vary with the dose of deferoxamine administered, body iron burden, and erythroid activity. Chelator-induced urinary iron excretion is also vulnerable to extraneous influences by infection, inflammation, the activity and effectiveness of erythropoiesis, extramedullary hematopoiesis, liver disease, and ascorbic acid deficiency.

**Serum ferritin**

Ferritin is found in virtually all cells, providing both an accessible reserve of iron for synthesis of functional iron-containing compounds and a means of sequestering iron in a soluble, apparently nontoxic form. It is especially abundant in cells with specialized roles in the synthesis of iron-containing compounds (erythroid precursors) and in iron metabolism and storage (macrophages, hepatocytes).

Intracellular ferritin is synthesized by the smooth endoplasmic reticulum in amounts required to replace catabolized ferritin and hemosiderin and to store any additional iron entering the cell. Small amounts of ferritin are also secreted into the plasma.Plasma ferritin is apparently synthesized by the rough endoplasmic reticulum

and glycosylated by the Golgi apparatus.

Under normal circumstances, the amount of plasma ferritin synthesized and secreted seems to be proportional to the amount of cellular ferritin produced in the internal iron storage pathway, so that the plasma ferritin concentration is related to the magnitude of body iron stores. The small amounts of ferritin secreted into the circulation can be measured by immunoassay and have a logarithmic relationship to body iron stores in normal individuals. In the absence of complicating factors, plasma ferritin concentrations decrease with depletion of storage iron and increase with storage iron accumulation.

Increased plasma ferritin concentrations may indicate increased iron stores, but a number of disorders may raise the plasma ferritin level independently of the body iron store. Plasma ferritin is an acute phase reactant, increased ferritin synthesis being a nonspecific response that is part of the general pattern of the systemic effects of inflammation. Thus, fever, acute infections, rheumatoid arthritis, and other chronic inflammatory disorders elevate the plasma ferritin concentration. Both acute and chronic damage to the liver, as well as to other ferritin-rich tissues, may increase plasma ferritin as an inflammatory process or by releasing tissue ferritins from damaged parenchymal cells; these tissue ferritins are not glycosylated.

De Virgilis[21] et al studied serum ferritin, liver iron stores, and liver histology in 38 children with thalassaemia major who were being treated by regular blood

transfusions. In the study it was noted that there was no correlation between serum ferritin levels and either the number of transfusions or the amount of iron deposited in the liver. However, for a given level of iron stores, ferritin levels were higher in patients with chronic hepatitis (including chronic aggressive and chronic persistent forms) than in those with hepatic siderosis only. It was concluded that serum ferritin reflects tissue iron deposits in regularly transfused thalassaemic patients, only in the absence of hepatitis.

In a study done by Letsky et al [22] the effect of iron chelation treatment on iron overload has been assessed by estimating serum ferritin levels and liver iron concentrations in both chelator-treated and control groups. When compared with non-chelated controls, results of both these estimations were invariably lower in the chelated group. However, all the results in both groups were very high, and fell within the ranges observed in untreated idiopathic haemochromatosis.They found a close correlation between serum ferritin levels and liver iron concentrations in regularly transfused children on continuous chelation therapy, indicating that serum ferritin is a valuable alternative to liver iron concentration in the assessment of visceral iron overload, even when massive tissue siderosis is present.

Bonkovsky et al[23] did a study and compared the results of imaging procedures with those of other noninvasive techniques and liver biopsies in 48 patients. Serum

ferritin and computed tomography or magnetic resonance scanning had 100% sensitivity in detecting hepatic iron overload more than fivefold above the upper limit of normal (greater than 10.7 µmol Fe/100 mg dry liver) but did not detect lesser degrees of iron overload reliably. Computed tomography and magnetic resonance imaging were more specific than ferritin (64% and 92% vs. 21%) in the detection of iron excess, more than five times the upper limit of normal. It was concluded that computed tomography or magnetic resonance scanning as currently usually used is not cost-effective in routine evaluation of iron overload, although these imaging procedures may play a role in patients in whom liver biopsy is contraindicated. Because of their low cost and ready availability, serum ferritin remain the preferred screening study for iron overload.

**MRI**

Gomori et al[24] in their study found that Quantitative MR imaging is a readily available noninvasive method for the assessment of hepatic iron concentration in iron-overloaded patients, reducing the need for needle biopsies of the liver.

**CT liver density**

In a study done by Bell et al[26] Computed tomography (CT) was performed to estimate the density of the hepatic and splenic parenchyma in 18 patients with hemochromatosis.They found an association between CT density and serum ferritin ($r = 0.72$, $p < 0.01$). The difference in density between liver and spleen gave better

discrimination between patients and controls: 12 of 18 (67%) showed an increased difference in density between liver and spleen.

A study was done by Howard et al[26] to determine whether or not hepatic computed tomography density is an alternative to liver biopsy for the diagnosis of body iron overload.Hepatic computed tomography density was determined in healthy controls, patients with idiopathic hemochromatosis, and patients with liver disease. An elevated hepatic computed tomography density associated with an elevated serum ferritin indicates iron overload; When computerized tomography is applied to patients with an unexplained elevation of the serum ferritin, it provides a noninvasive alternative to liver biopsy for the detection of excess hepatocellular iron.

JS Mitnick et al[27] reported the striking increase in lymph node density due to hemochromatosis observed with computed tomography (CT) in nine patients with Cooley anemia treated with multiple blood transfusions. The CT appearance and pathologic findings of hemochromatosis of the liver and spleen in three of these patients were also observed and correlated with pathologic specimens. CT density of the liver seemed to relate to the degree of hepatic fibrosis or cirrhosis, rather than the amount of iron.

Guvader et al[28] did a study to evaluate the effectiveness of single-energy computed tomography in determining iron overload in idiopathic hemochromatosis, with special reference to slightly overloaded cases. Liver attenuation was determined in 100 patients (46 cases of idiopathic hemochromatosis, 32 cases of chronic liver disease, and

22 normal controls). The iron load was determined for the first two groups by biochemical determination of liver iron concentration (performed in all but 12 subjects in the chronic liver disease group) and hepatic histologic grading. The main results for liver attenuation (upper normal limit, 72 Hounsfield units) showed that despite a high specificity (0.96), this parameter was of low sensitivity (0.63). Although mean liver attenuation in idiopathic hemochromatosis (77 +/- 14) was significantly higher than in chronic liver diseases (53 +/- 17; p less than 10(-4) and normal controls (66 +/- 3; p less than 10(-3], and despite an overall good correlation between liver attenuation and liver iron concentration (r = 0.72; p less than 10(-3], liver attenuation was unable to detect moderate iron overload. Fourteen of 18 patients with a liver iron concentration of less than 150 µmol/g dry liver wt had liver attenuation values of less than 72. Moreover, 3 of 18 subjects with a liver iron concentration of greater than 150 had a liver attenuation of less than 72. Of these 17 false-negatives, only 7 could be attributed to associated steatosis. On the whole, single-energy computed tomography, when used on a routine basis for diagnosing iron overload, is of limited clinical value in idiopathic hemochromatosis due to its poor sensitivity. Hepatic histologic examination together with biochemical determination remains the most accurate means to assess liver iron.

In a study done by Houang et al[29] Computer tomographic (CT) scans of the liver were obtained in six thalassaemic patients with iron overload confirmed by liver biopsy. Mean CT values for the liver in individual patients were linearly related to the iron content estimated by liver biopsy (correlation coefficient = 0.995).

In a study done by Babiker et al[30] thirty-seven children with beta-thalassaemia major, eight children with liver cirrhosis, and 20 matched controls were enrolled. Serum ferritin was determined in each subject by radio-immunoassay and liver enzymes by standard methods. The liver, spleen, kidney and pancreas densities were obtained by computed tomography. The iron content of liver biopsies from 10 patients was graded by staining. The mean serum ferritin of the thalassaemic patients was significantly higher than that of the control group ($p = 0.0001$). The ferritin of patients with cirrhosis and Wilson's disease was similar to that of the control group. The liver density of the thalassaemic patients was significantly higher than that of the control group (p less than 0.0001) while that of patients with liver cirrhosis and Wilson's disease was similar to the control group. The liver iron content of patients with liver cirrhosis was within the normal range. The spleen and kidney densities of patients with thalassaemia were higher than that of the control group with p values of 0.02 and 0.056, respectively. The density of the pancreas in patients with thalassaemia was not significantly different from that of the control group, ($p = 0.52$). There was correlation between the liver density and serum ferritin in patients with thalassaemia ($r = 0.432$, p less than 0.01) while there was no correlation between spleen, pancreas and kidney densities with serum ferritin.

In a study done by Guvader et al[31] it was found that Serum iron and transferrin saturation are poorly correlated with the degree of iron overload. Serum ferritin is a better reflect of iron stores but numerous clinical conditions, unrelated to variations of iron load, can increase the serum level. Biochemical determination of liver iron overload

is the gold standard of iron quantification and well correlated to the level of iron burden appreciated by the amount of iron removed by venesection, but its determination necessitates a liver biopsy and is dependant of sampling error in case of heterogeneous iron deposits (cirrhosis). The sensitivity of computed tomography is insufficient, being unable to detect iron overload below 5 times the normal liver iron load, especially in case of associated steatosis. Magnetic resonance imaging is a valuable tool when using T2 weighted gradient echo sequences on 1.5 Tesla magnet and permits non invasive iron overload quantification.

In the study done by Gomori et al, the authors assessed the use of T2 measurements obtained by means of MR imaging as well as computed tomographic (CT) attenuation as a measure of liver iron concentration in 10 severely iron-overloaded patients with beta- thalassemia major.

The iron concentrations in surgical wedge biopsy samples of the liver, which varied between 3 and 9 mg/g of wet weight (normal, less than or equal to 0.5 mg/g), correlated well (r = .93, P less than or equal to .0001) with the preoperative hepatic T2 measurements. The CT attenuation did not correlate with liver iron concentration.

In a study done by Bakdekar et al[32] it was shown that significant correlation exists between serum ferritin and CT liver density.

# AIM OF THE STUDY

- To determine the correlation between serum ferritin and CT liver density in transfused Thalassemia major children.

# MATERIALS AND METHODS

- Setting

  Study was conducted in institute of child health and research centre-Government Rajaji hospital,Madurai.

- Collaborarion Departments

  The study was done in collaboration with Department of Radiology and Department of Biochemistry, Madurai.

- Ethical committee

  Approval for the study was obtained from The Ethical committee of Govt.Rajaji Hospital.

- Study design

  Prospective cross sectional analytical study

- Study period

  The study period was from January 2007 to June 2008

- Sample size

  A total of 30 children which included all Thalassemia major children enrolled

in Institute of child health and research centre, Government Rajajii Hospital, Madurai.

- Inclusion and Exclusion criteria:

All Thalassemia major children upto 12 years enrolled were included and none of the children were excluded.

## PROCEDUR

Ethical approval was obtained.

- Relevant information was gathered using proforma.
- 5 ml of venous sample was collected for serum ferritin estimation.
- Immunoenzymatic calorimetric method was used for quantitative determination of serum ferritin.
- The ferritin concentration in the sample was calculated based on a series of standard.
- The colour intensity was proportional to the ferritin concentration in the sample.
- CT abdomen was done to determine liver density.It was done on the same on the day of collection of blood sample for serum estimation.
- Five readings of liver density were determined in each lobe avoiding biliary tract, portal vein and porta hepatis.An average of these ten readings was designated as

liver density of the patient.

- Liver biopsy was done for four patients.

## STATISTICAL ANALYSIS:

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using Epidemiological Information Package (EPI 2002).

Using this software, frequencies, percentages, means, standard deviations, chi square, ‘p’ and coefficient of correlation values were calculated. Kruskul Wallis chi-square test was used to test the significance of difference between quantitative variables and Yate’s test for qualitative variables. A 'p' value less than 0.05 is taken to denote significant relationship. If the coefficient of correlation (r) is more than 0.5 then the two variables are taken to be correlated.

## OBSERVATIONS

TABLE 1 : AGE DISTRIBUTION

Total number of children enrolled in the study were 30. 53.3% of the children were between 2-5 years of age.30% were between 6-8 years of age.10% were between 9-10 years of age.6.7% were upto 2 years of age.

| Age group | Cases | |
|---|---|---|
| | **No** | **%** |
| Upto 2 years | 2 | 6.7 |
| 2-5 years | 16 | 53.3 |
| 6-8 years | 9 | 30 |
| 9-10 years | 3 | 10 |
| Total | 30 | 100 |
| Mean<br>S.D. | 5.13 years<br>2.48 | |

**TABLE 2: SEX DISTRIBUTION**

| Sex | Cases | |
|---|---|---|
| | **No** | **%** |
| Male | 20 | 66.7 |
| Females | 10 | 33.3 |
| Total | 30 | 100 |

- 66.7% of 30 chidren were males.33.3% of children were females.

**TABLE 3 : CONSANGUINITY**

| CONSANGUINITY | Cases | |
|---|---|---|
| | No | % |
| Second degree | 8 | 26.7 |
| Thirddegree | 8 | 26.7 |
| No Consanguinity | 14 | 45.7 |

53.4% of children studied were born of consanguinous parents.45.7% of children studied were born of nonconsanguinous parents.

**TABLE 4 : HEMOLYTIC FACIES**

| Hemolytic facies | Number of children | % |
|---|---|---|
| Present | 16 | 53.3 |
| Absent | 14 | 46.7 |

53.3% of children studied had hemolytic facies. 46.7% of children did not have hemolytic facies.

**TABLE 5 : REGULARITY OF TRANSFUSION**

| Regularity of | Number of children | % |
|---|---|---|

| transfusion | | |
|---|---|---|
| Regular | 18 | 60 |
| Irregular | 12 | 40 |

Out of 30 children studied 60% had regular transfusion and 40 % were not on regular transfusion.

**TABLE 6 : NUMBER OF TRANSFUSIONS,CT LIVER DENSITY AND SERUM FERRITIN**

| | Range | Mean | SD |
|---|---|---|---|
| V. Number of Transfusions | 8-108 | 34.9 | 20.5 |
| VI. C.T. liver Density | 36-112 ng/ml | 81.7 | 20.5 |
| VII. Serum Ferritin | 220-1750 HU | 780 | 360 |

Number of transfusions ranged from 8-108.CT liver density ranged from 36-112HU with a mean of 81.7HU.serum ferritin ranged from 220-1750ng/ml with a mean of 780 ng/ml.

Table 7 : LIVER DENSITY AND SERUM FERRITIN

| Serum Ferritin | Liver Density | | |
|---|---|---|---|
| | Range | Mean | S.D. |
| Upto 250 (1) | 38 | 38 | - |
| 251-500 (4) | 36-60 | 48 | 9.8 |
| 501-750 (11) | 66-89 | 77 | 7.7 |
| 751-1000 (9) | 88-98 | 94.4 | 3.4 |
| > 1000 (5) | 102-112 | 106 | 4.2 |
| Total | 36-112 | 81.9 | 20.5 |
| 'p' | 0.0001 Significant | | |

Out of 30 children about 11 of the children had serum ferritin ranging from 501-750 ng/ ml and their liver density were between 66-89 HU. 9 children had serum ferritin between 751-1000 ng/ml and their liver density were between 88-98 HU.5 had serum ferritin more than 1000 ng/ml .Their liver density were between102-112 HU.4 children had serum ferritin between 251-500 ng/ml and liver density between 36-60 HU. 1 had serum ferritin less than 250 and had liver density of 38.

**TABLE 8 : CORRELATION OF COEFFICIENT BETWEEN SERUM FERRITIN AND CT LIVER DENSITY**

| | Serum ferritin and CT liver density |
|---|---|
| Coefficient of correlation | 0.8526 |

Stastically significant correltion was found between serum ferritin and CT liver density.

**TABLE 9 : NUMBER OF TRANSFUSIONS AND**

**CT LIVER DENSITY**

| Number of transfusions | CT liver density | |
|---|---|---|
| | Mean | SD |
| UPTO 20 | 56.75 | 16.59 |
| 21-40 | 83.82 | 11.85 |
| 41-60 | 96.78 | 9 |
| >60 | 105 | 4.24 |
| P | 0.0003 SIGNIFICANT | |

12 children had 20-40 transfusions .Their mean CT liver density was 83. 82.8 childen had transfusions ranging from 8-20 and their mean CT liver density was 56.75.Another 8 children had 41-60 transfusions .Their mean CT liver density was 96.78 HU. 2 of the children had more than 60 transfusions and their mean CT liver density of 105 HU.

**TABLE 10 : CORRELATION OF COEFFICIENT BETWEEN NUMBER OF TRANSFUSIONS AND CT LIVER DENSITY**

| | Number of transfusions and CT liver density |
|---|---|
| Coefficient of correlation | 0.7706 |

Stastically significant correlation was found between number of transfusions and CT liver density

**TABLE 11 : SPLENOMEGALY**

| At the time of diagnosis | Number of children | % |
|---|---|---|
| Mild | 14 | 48.7 |
| Moderate | 12 | 41.4 |
| Massive | 3 | 10.3 |
| **At the time of study** | | |
| Mild | 7 | 24.1 |
| Moderate | 18 | 62.1 |
| Massive | 4 | 13.8 |

48.7% of children had mild splenomegaly, 41.4% had moderate and 10.3% had massive splenomegaly at the time of diagnosis.During present study ,62.1% had moderate splenomegaly,24.1% had mild splenomegaly and 4% had massive splenomegaly.

**TABLE 12**

**REGULARITY OF TRANSFUSION AND SPLEEN SIZE**

| Regularity of transfusion | Spleen size | | | | | |
|---|---|---|---|---|---|---|
| | No change | | Decrease | | Increase | |
| | No. | % | No. | % | No. | % |
| Regular (18) | 14 | 77.8 | 2 | 11.1 | 2 | 11.1 |
| Irrregular (11) | 2 | 18.2 | - | - | 9 | 81.8 |
| 'p' | 0.0002 Significant | | | | | |

Out of 18 children who had regular transfusion 77.8% had no change in spleen size.11.1% had decrease in spleen size and another 11.1% had increase in spleen size.18.2% of 11 children who had irregular transfusion had no change in spleen size . 81.8% had increase in spleen size.

**TABLE 13 : SPLENOMEGALY AND SERUM FERRITIN**

| Splenomegaly | Serum ferritin | |
|---|---|---|
| | Mean | SD |
| a) At diagnosis | | |
| Mild | 744.7 | 320.8 |
| Moderate | 929.5 | 399.7 |
| Massive | 495.3 | 243 |
| 'p' | 0.1021 | |
| | Not significant | |
| b)At the time of study | | |
| Mild | 616.6 | 189.8 |
| Moderate | 852.1 | 368.6 |
| Massive | 853.5 | 551.8 |
| 'p' | 0.3171 | |
| | Not significant | |

Correlation between splenomegaly and serum ferritin was not stastically significant

## TABLE 14 : SPLENOMEGALY AND CT LIVER DENSITY

| Spleenomegaly | Liver density | |
|---|---|---|
| | Mean | SD |
| a) At diagnosis | | |
| Mild | 79.1 | 18.4 |
| Moderate | 89.8 | 20 |
| Massive | 62.7 | 7 |
| 'p' | 0.0713 Not significant | |
| b)At the time of study | | |
| Mild | 72.3 | 19.4 |
| Moderate | 86.1 | 18.7 |
| Massive | 79.5 | 31.6 |
| 'p' | 0.3309 Not significant | |

No correlation was established between spleenomegaly and CT liver density.

# DISCUSSION

The creation of excessive iron stores associated with hemosiderosis is a major concern in individuals with beta Thalassemia major.They develop iron overload as a result of their inability to excessive iron which they receive via multiple blood transfusions.Increased iron absorption also contributes to iron excess. Managing iron overload in Thalassemia syndromes require a reliable assessment of excessive iron overload and organ iron distribution. Measurement of hepatic iron concentration is the most quantitative, specific, and sensitive method for determining the body iron burden in patients with thalassemia major.

Liver biopsy is the best direct means of assessing iron deposition, permitting chemical measurement of the nonheme (storage) iron concentration and histochemical examination of the pattern of iron accumulation in hepatocytes and Kupffer cells as well as evaluation of the extent of inflammation, fibrosis, and cirrhosis. This direct method for assessing iron status have the disadvantages of being invasive procedures, with their attendant discomfort, lack of acceptability to patients, and, in the case of liver biopsy, risk. Several noninvasive means of measuring tissue iron stores are under development, including determination of hepatic magnetic susceptibility, computed tomography (CT),and magnetic resonance imaging (MRI).CT abdomen was used in the present study as it is widely used and less expensive.

A total of 30 thalassemia major children were studied to determine the usefulness of CT abdomen in determining iron overload.

Out of the 30 children included in this study 53.3 % were in the age group between 2-5 yearsof age,6.7% were upto 2 years of age,30% of children between 6-8 years of age and 10% of children between 9-10 years of age.Mean age in the present study was 5.13 years.

In a study done by Bhaswati et al[4] it was found that 84.2% of the patients attending govt. hospitals were below 10 years of age as compared to only 48% attending the Thalassemia Society in the same age group. The difference was found to be statistically significant ($p<0.05$).

In 1973, a survey was done of the ages of 243 living patients with thalassemia major followed at 12 centers in the United States and Canada. Twenty-two percent were younger than 5 years and 2.1% were older than 25 years of age (mean 11.4 ± 6.7 [SD] years). In 1985, there were 303 patients at the same centers; 11% were younger than 5 years and 7.9% were older than 25 years (mean 14.2 ± 7.3 years).

66.7% of the children in the study were males and 33.3% were females.In the study done by Bhaswati et al has also found that in both govt. as well as NGO sector most of the thalassemic patients were male, being 58.6% and 72% respectively.

26.7% of children were born of first degree consanguinity and 26.7% of children were born of second degree consanguinity.53.3 % of children had hemolytic facies.

60% of children had regular transfusion whereas 40% of children had irregular transfusion.

Number of transfusions given varied from 8-108.serum ferritin levels ranged from 220-1750 nano gm/ml.Average serum ferritin was 780 ng/ml. CT liver density ranged from 36-112 HU and the mean CT liver density was 81.7 HU.

Melody et al has done a study in 342 beta thalassemia patients and serum Ferritin levels ranged from 147 to 11 010 ng/mL (median, 1696 ng/mL).In the study done by Bakdekar et al to study the correlation between serum ferritin and CT liver density, serum ferritin ranged from 1190 ng/ml to 15,000 ng/mland average serum ferritin level was 4289.4 ng/ml.CT liver density ranged from 72.3-119.8 hounsefield units and the mean was 98.

Out of 30 children11 HU children had serum ferritin between 501-750 ng/ml had CT liver density between 66-89 HU.9 children had serum ferritin between 751-1000 ng/ml and their liver density were between 88-98 HU.5 had serum ferritin more than 1000 ng/ml .Their liver density were between102-112 HU.4 children had serum ferritin between 251-500 ng/ml and liver density between 36-60 HU. One child had serum

ferritin less than 250 and had liver density of 38.

Significant correlation was between serum ferritin and CT liver density and coefficient of correlation was 0.8526.

Out of the 30 children studied 12 children had 20-40 transfusions .Their mean CT liver density wss 83. 82.8 childen had transfusions ranging from 8-20 and their mean CT liver density was 56.75.Another 8 children had 41-60 transfusions .Their mean CT liver density was 96.78 HU.2 of the children had more than 60 transfusions and their mean CT liver density was 105 HU.

In a study done by Houang et al[29] Computer tomographic (CT) scans of the liver were obtained in six thalassaemic patients with iron overload confirmed by liver biopsy. Mean CT values for the liver in individual patients were linearly related to the iron content estimated by liver biopsy (correlation coefficient = 0.995).

Significant correlation was found between number of transfusions and CT liver density .Coefficient of correlation was 0.7706.

In a study done by Guvader et al[28] to evaluate CT in the assessment of iron overload the main results for liver attenuation (upper normal limit, 72 Hounsfield units) showed that despite a high specificity (0.96), this parameter was of low sensitivity

(0.63).

In a study done by Howard et al[26], hepatic computed tomography density ranged from 11 to 36 units (mean = 30)in 69 controls. Given an upper limit of normal of 36 computed tomography units, 4 of 6 untreated patients with hemochromatosis had elevated hepatic computed tomography density.Hepatic computed tomography density correlated directly with serum ferritin ($r = 0.72$, $p = 0.01$). In 58 consecutive patients with clinical or biochemical evidence, or both, of liver disease who underwent liver biopsy for diagnostic purposes, 0 of 52 patients with normal hepatic iron had an elevated hepatic computed tomography density as compared with 4 of 6 patients with excess iron ($X2 = 35$, p less than 0.001).

In a study done by Bell et al[25] Computed tomography (CT) was performed to estimate the density of the hepatic and splenic parenchyma in 18 patients with hemochromatosis. The mean CT density was 79 +/- 21 Hounsfield units compared with 61 +/- 9 ($p < 0.01$) in 31 controls without hepatic disease. Increased density above 79 Hounsfield units was found in eight patients out of 18 (44%). The highest density (125 Hounsfield units) was found in a patient with a serum ferritin of 6500 micrograms/l. They found an association between CT density and serum ferritin ($r = 0.72$, $p < 0.01$).

Liver biopsy was done in 4 children.one child had hemosiderosis without evidence of cirrhosis.He had 38 blood transfusions and had serum ferritin of 860 ng/ml.His liver density was 96 HU.

Two children had hemosiderosis with cirrhosis.One had 108 transfusions His serum ferritin was 1182 ng/ml and liver density was 104 HU.Another had 90 transfusions.His serum ferritin was 1160 ng/ml and liver density was 112 HU.

One child had hemosiderosis with periportal fibrosis in liver biopsy.There was no evidence of cirrhosis.She had 42 transfusions.Her serum ferrritin was 920 ng/ml and liver density was 96 HU.

Bonkovsky et al[1] did a study and compared the results of imaging procedures with those of other noninvasive techniques and liver biopsies in 48 patients. Serum ferritin and computed tomography or magnetic resonance scanning had 100% sensitivity in detecting hepatic iron overload more than fivefold above the upper limit of normal (greater than 10.7 mumol Fe/100 mg dry liver) but did not detect lesser degrees of iron overload reliably. Computed tomography and magnetic resonance imaging were more specific than ferritin (64% and 92% vs. 21%) in the detection of iron excess, more than five times the upper limit of normal. It was concluded that computed tomography or magnetic resonance scanning as currently usually used is not cost-effective in routine

evaluation of iron overload, although these imaging procedures may play a role in patients in whom liver biopsy is contraindicated. Because of their low cost and ready availability, serum ferritin remain the preferred screening study for iron overload.

48.7% of children had mild splenomegaly, 41.4% had moderate and 10.3% had massive splenomegaly at the time of diagnosis.During present study, 62.1% had moderate splenomegaly, 24.1% had mild splenomegaly and 4% had massive splenomegaly.

Out of 18 children who had regular transfusion 77.8% had no change in spleen size.11.1% had decrease in spleen size and another 11.1% had increase in spleen size.18.2% of 11 children who had irregular transfusion had no change in spleen size . 81.8% had increase in spleen size.

# CONCLUSIONS

1) Males are more commonly affected than females.

2) 45.7% of thalassemia major children are born to non consanguinous parents.

3) Only 60% of affected children are on regular transfusions.

4) Significant correlation exists between serum ferritin and CT liver density.

5) Children with cirrhosis had serum ferritin more than 1000 ng/ml and CT liver density more than 100 HU.

6) Significant correlation exists between CT liver density and number of transfusions.

7) Spleen size regresses with regular transfusion.

8) No correlation exists between serum ferritin and splenomegaly.

9) There is no correlation between CT liver density and splenomegaly.

# LIMITATIONS OF THE STUDY

- Small sample size.
- Liver biopsy being an invasive procedure consent could be obtained only for 4 cases.
- Quantitative iron estimation in liver biopsy could not be done.

# RECOMMENDATIONS

1) CT may be used for estimation of iron overload in thalassemia major children.

2) To improve the regularity of transfusions day care facility should be established.

3) Antenatal screening should be done for all mothers who are NESTROF test positive irrespective of consanguinity.

4) Iron chelation should be started at serum ferritin less than 1000ng/ml in contrast to earlier studies and CT liver density less than 100 HU to prevent cirrhosis liver.Further studies are needed.

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# PROFORMA

NAME :

AGE :

SEX :

ADDRESS :

FATHER'S NAME: MOTHER'S NAME:

CONSANGUINITY:

| COMPLAINTS | YES | NO | DURATION |
|---|---|---|---|
| Easy fatiguability | | | |
| Breathlessness,pedal edema | | | |
| Abdominal distension | | | |
| Jaundice | | | |
| Skin pigmentation | | | |
| Failure to thrive | | | |
| Frequent infections | | | |
| Pica | | | |

PAST HISTORY:

| | |
|---|---|
| H/O hospitalization | |
| Number of blood transfusions | |
| Transfusion since the age of | |

IMMUNISATION HISTORY:

Hepatitis B vaccination

FAMILY HISTORY:

| | YES | NO | DURATION |
|---|---|---|---|
| Parents affected | | | |
| Siblings affected | | | |

ANTHROPOMETRY:

| | |
|---|---|
| Height | Weight |
| HC | MAC |

GENERAL EXAMINATION:

| | YES | NO |
|---|---|---|
| Pallor | | |
| Icterus | | |
| Pedal edema | | |
| Lymphadenopathy | | |
| Hemolytic facies | | |
| Bossing of skull | | |
| Malformed tooth | | |
| Kolionychia | | |
| Skin pigmentation | | |

| PER ABDOMEN: |
|---|
| Distension: |
| Hepatomegaly: |
| Splenomegaly: |
| Free fluid: |

| CARDIOVASCULAR SYSTEM | RESPIRATORY SYSTEM |
|---|---|
| Heart sounds | Trachea |
| Murmurs | Air entry |
| | Added sounds |

| CENTRAL NERVOUS SYSTEM: |
|---|
| Cranial nerves: |
| Motor system: |
| Sensory system: |

**INVESTIGATIONS :**

Hemoglobin :

Peripheral smear :

Hemoglobin electrophoresis:

Serum ferritin :

CT liver density :

Liver biopsy :

## ABBREVIATIONS

1) CT - Computed Tomography

2) MRI - Magnetic Resonance Imaging

3) HU - Hounsfield Unit

4) SD - Standard Deviation

5) Hb - Hemoglobin